GENETIC VARIATION IN THE 16S MITOCHONDRIAL rDNA GENE FROM TEXAS AND OKLAHOMA POPULATIONS OF *Amblyomma maculatum*

A Thesis

by

TRACY KARON LOSTAK

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2008

Major Subject: Entomology

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Approved by:

Chair of Committee,	Pete Teel
Committee Members,	Craig Coates
	Michael Longnecker
Head of Department,	Kevin Heinz

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ABSTRACT

Genetic Variation in the 16S Mitochondrial rDNA Gene from Texas and Oklahoma Populations of *Amblyomma maculatum***.** (August 2008)

Tracy Karon Lostak, B.S., Sam Houston State University

Chair of Advisory Committee: Dr. Pete Teel

Single-strand conformation polymorphism was used to detect different haplotypes of the 16S mitochondrial rDNA gene within samples of Gulf Coast ticks, *Amblyomma maculatum* Koch, collected from Payne County, Oklahoma and Brazos and Refugio Counties, Texas. Ticks were collected from dogs, horses, and cattle. The haplotype frequencies from the cattle collections were compared to a similar study, conducted in 1999, to detect if any changes in frequencies had occurred. There were significant differences ($p<0.05$) between the haplotype frequencies from 1999 and 2007. The haplotype designated as D was highly prevalent in all sampled populations, however was not detected in Oklahoma and Texas eight years earlier. Possible explanations for this occurrence include ticks with this haplotype having a higher fecundity, resistance to drought, or resistance to acaricides. Comparisons of the haplotypes of ticks collected from cattle solely in 2007 showed that the haplotype frequencies of Brazos County and Payne County are more similar than to Refugio County. The haplotype frequencies found on various hosts were also compared and no significant differences were found ($p>0.05$).

DEDICATION

To my husband and parents

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my husband, Gleb, who has supported and encouraged me every step of the way. Watching you complete your dissertation helped me when it came to writing my thesis. If it weren't for you, this experience would have been much more stressful and difficult.

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I would also like to thank Otto Strey for all the support and help you have given me. You not only collected ticks for me, but you were always there if I was having problems with the protocols and you made sure I had everything needed for the project. You have been a tremendous help and if it wasn't for you I would probably still be grinding tick legs.

Paul Barron, we have never met but this project might not have been possible if it wasn't for your time and effort spent on setting up the procedures I eventually used.

Finally, I would also like to thank everyone who participated in collecting ticks for this project. Dr. Thomas Craig, from the Department of Pathobiology at Texas A&M College of Veterinary Medicine, who brought us the ticks collected from dogs. Lisa Coburn, from the Department of Entomology and Plant Pathology at Oklahoma State University, who collected the ticks from the Lake Carl Blackwell Research Station. Phil Kaufman, from the Department of Entomology and Nematology at the University of Florida, who collected ticks from Nassau, Marion, and Alachua Counties in Florida. Wallace and Phillip Shay, who allowed us access to Shay Ranch and Victor Pineda, who collected the ticks from horses at the Texas A&M Riverside Campus.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

The Gulf Coast tick, *Amblyomma maculatum* Koch, is a three-host tick within the family Ixodidae. It was first described in 1844 and has always held a significant status as a pest of livestock, however only recently has it gained status as a vector of pathogens such as *Rickettsia parkeri* Lackman et al. 1965*, Ehrlichia ruminantium* Dumler, formerly *Cowdria ruminantium,* and *Hepatozoon americanum* Vincent-Johnson et al. 1997. As more is known about *A. maculatum* ecology and its potential links to diseases in humans, cattle, and dogs, associations can be made between genetic variation within the species and vector competence and capacity.

Amblyomma maculatum is primarily distributed along the areas bordering the Gulf of Mexico and the Atlantic Ocean (Figure 1) however there are established populations inland in Oklahoma and Kansas (Bishopp and Trembley 1945; Semtner and Hair 1973; Goddard and Norment 1983). Established populations also exist in Central and South America and islands of the Caribbean.

The life cycle of this species was first documented by Hixson (1940). A gravid female *A. maculatum* will oviposit in a safe location off the host, usually a moist area in the soil-vegetation interface. The incubation period of the eggs ranges from 19 to 28 days, but this period can be longer if the eggs are deposited near the end of September due to cooler temperatures. Once larvae have emerged, they tend to gather near the lower

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This thesis follows the style of the Journal of Medical Entomology.

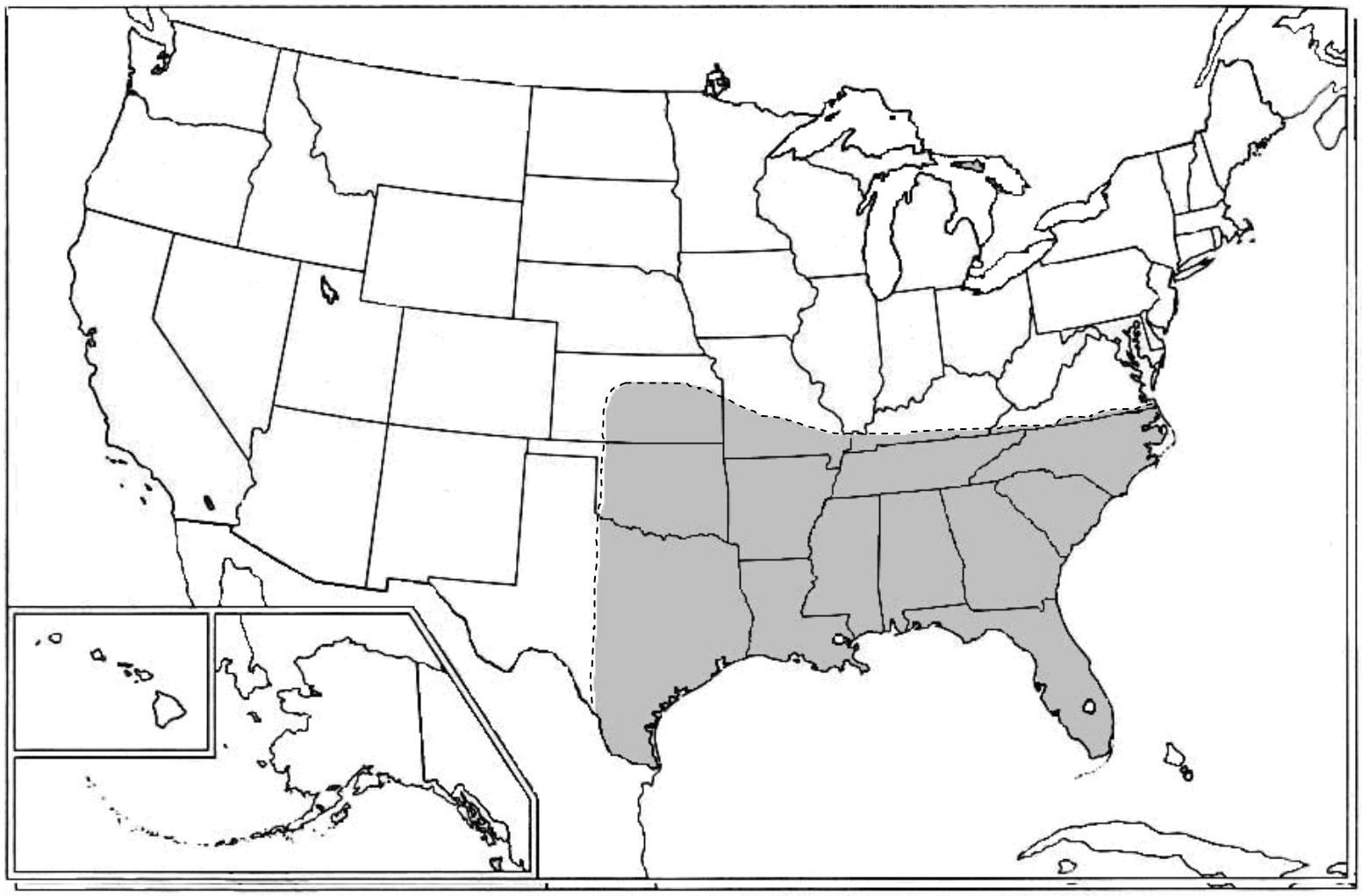

Fig. 1. General distribution of *Amblyomma maculatum* in the United States (shaded region) as interpreted from Bishopp and Trembley (1945), Semtner and Hair (1973), Goddard and Norment (1983), and Sonenshine et al. (1965).

surface of stems or leaves and will stay inactive for a period of days**.** If larvae sense the presence of a host, they will crawl to the top of the stem and extend their forelegs in preparation for attachment to the host. Once attached, larvae will engorge within seven days then drop from the host and hide themselves on the ground near low vegetation and then molt.

Nymphal host seeking activity is relatively the same as larva. They remain hidden until they sense the presence of a host, then crawl to the top of the vegetation, attach to the host, engorge, and drop from the host to hide in the vegetation where they molt into adults. Mating between adults occurs on the host and then gravid females will drop from the host and oviposit their eggs.

The developmental time for the *A. maculatum* life stages can vary depending on temperature and vegetation (Fleetwood 1985). Larva and adults associated with mixed brush habitats require the longest developmental time whereas those associated with buffelgrass habitats are much shorter. Also, developmental time will be faster during periods with high average daily temperatures.

There are differences in the timing of the peak abundance of larva, nymphs, and adults between coastal populations, such as those along the Texas Gulf Coast, and inland populations, in Oklahoma and Kansas. Adult inland populations are active five months earlier than their coastal counterparts (Teel et al. 1998, Semtner and Hair 1973). In the coastal regions of Texas, larval and nymph peak activity occurs in January and February followed by peak adult feeding in September (Hixson 1940, Teel et al. 1998). In Oklahoma and Kansas, peak abundance of larvae and nymphs occurs in July and August and adult peak abundance occurs in May through June (Semtner and Hair 1973).

Amblyomma maculatum utilizes a variety of host species. The larvae and nymphs are known to feed on rodents and ground dwelling birds and can usually be found attached to the host's neck and head. Adult *A. maculatum* and nymphs feed upon medium to large mammals such as cattle, horses, dogs, goats, and wolves and they can frequently be found in the inner and outer surface of the host's ear (Figure 2, Bishopp and Trembley 1945). The attachment of the tick can cause scabbing and cracking of the skin, induce muscle damage, and increase the risk of bacterial infections (Riley et al. 1995).

The Gulf Coast tick is a common pest found on livestock and can predispose animals to primary and secondary screwworm infestations. The long mouthparts of these ticks and their feeding in close proximity to each other produces significant lesions on the skin and can cause the ear to swell. This can prevent the animal from flicking the ear and allowing *Cochliomyia* flies, which are attracted to lesions on the skin, to oviposit with ease (Bishopp and Hixson 1936). The United States and Mexico have eliminated primary screwworm through eradication programs, however there is always a danger of reintroduction of the species due to natural populations in South America and through the international trade of livestock and increased international travel. Secondary screwworm is still present in the U.S and is considered an important pest although they do not feed on living tissue.

In recent years, the Gulf Coast tick has become associated with three pathogenic agents. *Rickettsia parkeri* has been associated with the Gulf Coast tick since 1939 and was shown to cause a mild febrile disease in guinea pigs that resembled spotted fever rickettsiosis, which are symptoms of other known pathogenic *Rickettsia* (Parker et al. 1939, Parker 1940). Speculation over the pathogenicity of *R. parkeri* in humans has

Fig. 2. *Amblyomma maculatum* found on the surface of a host's ear.

occurred since its discovery. For years *R. parkeri* was believed to be a nonpathogenic endosymbiont of *A. maculatum* due its lack of association with clinical disease in humans however in 2002 the pathogen was identified from a man exhibiting symptoms associated with spotted fever rickettsiosis, including fever and a spotted rash (Goddard 2001, Paddock et al. 2004). In 2007, *R. parkeri* was isolated from a man with symptoms of rickettsiosis who could confirm being bitten by a tick that looked similar to *A. maculatum* (Whitman et al. 2007).

The prevalence of *R. parkeri* in humans is mostly unknown because there is a significant chance that some patients identified as having Rocky Mountain Spotted Fever (RMSF), which is caused by *Rickettsia rickettsii*, actually were infected with *R. parkeri*. This results from the fact that the diagnostic procedures are not species specific. Raoult and Paddock (2005) analyzed serum specimens for *R. rickettsii*, *Rickettsia akari*, and *R. parkeri* of patients that were previously diagnosed with RMSF and found 5 out of 15 samples that were positive for *R. parkeri*.

Rickettsia parkeri has been isolated from *A. maculatum* in Alabama, Florida, Georgia, Kentucky, Mississippi, Oklahoma, South Carolina, and Texas (Parker et al. 1939; Parker 1940; Sumner et al. 2007). This suggests *R. parkeri* can be found anywhere Gulf Coast ticks are located and means any people situated in areas infested with Gulf Coast ticks are at risk to infection and disease (Figure 1).

Gulf Coast ticks have also been shown to experimentally vector *Ehrlichia ruminantium*, a rickettsial pathogen causing the fatal heartwater disease in African domestic and wild ruminants (Uilenberg 1982; Mahan et al. 2000). *Ehrlichia ruminantium* has been found in the Caribbean, although it appears to be isolated to

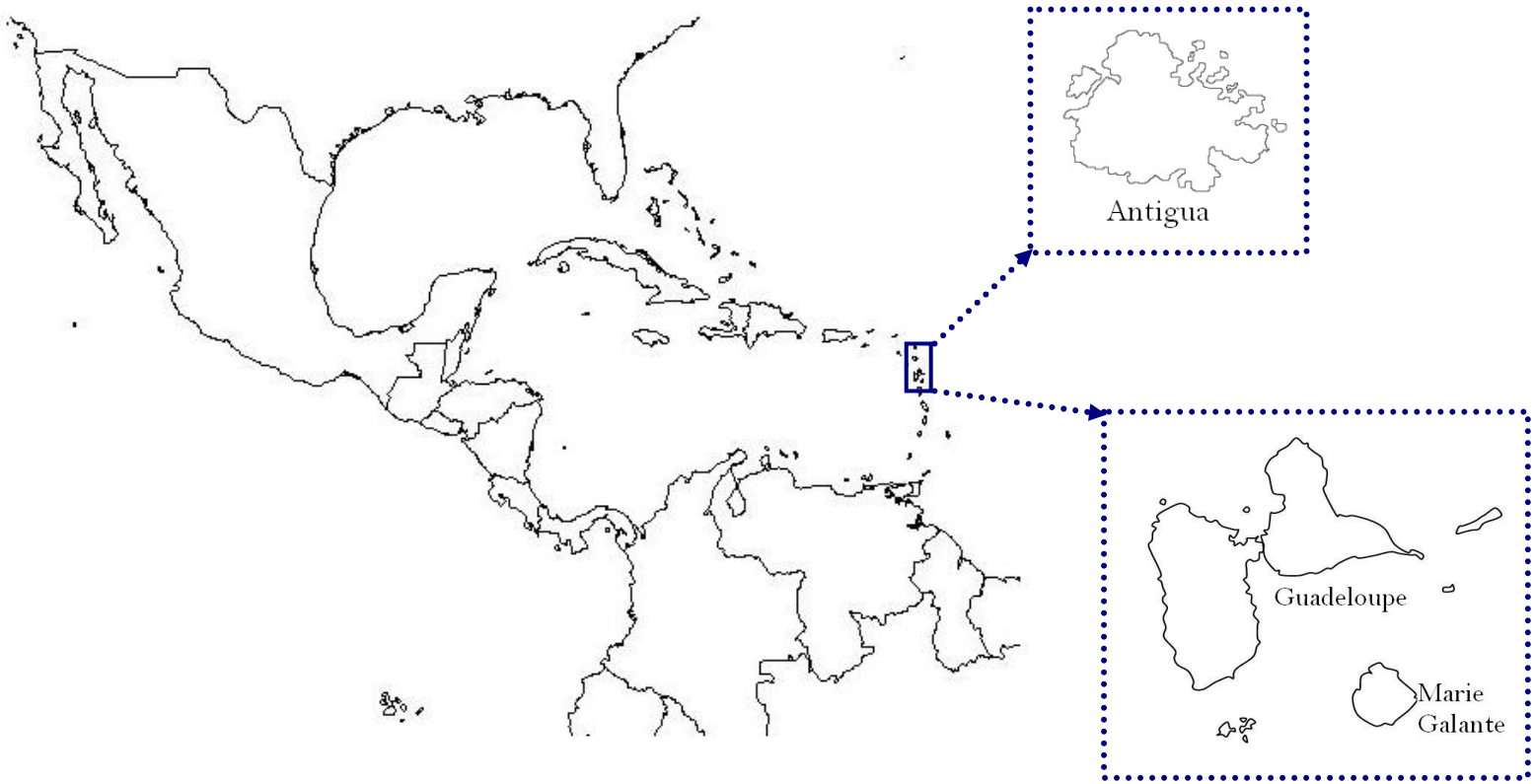

Fig. 3. Antigua, Guadeloupe, and Marie Galante are Caribbean islands with a known presence of heartwater, *Ehrlichia ruminantium*.

Guadeloupe, Marie Galante, and Antigua (Figure 3). *Amblyomma variegatum,* the tropical bont tick, is an African vector for *E. ruminantium* and has dispersed throughout much of the Caribbean. Climate matching models used to determine the suitability of the New World to the spread of *A. variegatum* suggest potential new areas suitable to this vector include the rest of the Caribbean, parts of Brazil, large areas of Columbia and Venezuela, Mexico, and the Florida Peninsula (Estrada-Peña et al. 2007). There is a potential risk of the pathogen and African vector entering the United States from the Caribbean by tick-infested migrating birds (Barré et al. 1987). Once in the U.S. there is a high risk of *A. maculatum* becoming a vector of the disease. *Amblyomma maculatum* experimentally infected at the larval and nymph stage have been shown to transmit the disease once they become an adult but they were not able to transmit it transovarially (Uilenberg 1982, Mahan et al. 2000). Also, *A. maculatum* has been shown to be highly susceptible to and a highly efficient vector of *E. ruminantium*. This tick's susceptibility to *E. ruminantium* equals that of the 2 major vectors, *A. variegatum* and *A. hebraeum*, and its ability to transmit the disease matches that of *A. variegatum* (Mahan et al. 2000). If *E. ruminantium* was ever introduced into the U.S. then *A. maculatum* would likely play a critical role in the maintenance and transmission of the disease, not only because of its ability to harbor and transmit the pathogen, but also because of its distribution (Figure 1) and prevalence on livestock and wildlife (Bishopp and Trembley 1945).

Heartwater is a serious disease that can cause death in 1 to 2 weeks. Recovery from the disease is rare without antibiotic treatment and no common vaccine for the different strains of *E. ruminantium* exists. For populations that are immunologically naïve, the case-fatality rate can be as high as 80% in cattle and even 100% for some

sheep and goat populations (Uilenberg 1983). The high fatality rate for this disease could lead to a large economic loss and possibly overwhelm some large animal veterinary clinics if it ever reaches the U.S.

Amblyomma maculatum also has the capacity to vector a protozoan that causes disease in dogs. *Hepatozoon americanum* is the primary agent of hepatozoonosis, which is an emerging canine disease in the United States. *Amblyomma maculatum* has been reported to be able to become infected with *H. americanum* at the larval and nymphal stages and produce viable oocysts once the tick is an adult. The disease is then transmitted to the dog when it eats the infected tick, possibly while grooming (Mathew et al. 1998; Ewing et al. 2002). Hepatozoonosis can often be severe and will frequently cause symptoms including fever, weight loss, lethargy, muscle atrophy, bloody diarrhea, bone pain, essentially symptoms associated with chronic inflammatory disease.

Other pathogenic agents have been isolated from *A. maculatum* including *Francisella tularensis* the causative agent for tularemia and Panola Mountain *Ehrlichia* (Blount 2007). It has been suggested that Panola Mountain *Ehrlichia* is a divergent strain of *E. ruminantium* and has been known to cause illness in goats and has been associated with illness in humans (Loftis et al. 2006, Reeves et al. 2008). It is not known whether *A. maculatum* is capable of serving as a vector for these two pathogens.

The Gulf Coast tick's ability to vector *Rickettsia parkeri, Ehrlichia ruminantium,* and *Hepatozoon americanum* and the fact that it has been shown to harbor *Francisella tularensis* and Panola Mountain *Ehrlichia* give cause to study the genetic variation within this species. Populations showing genetic similarity for certain chromosome markers are likely to have other similar traits including the ability to vector a disease (Tabachnick

1991). It is already known that inland populations of *A. maculatum* in Oklahoma and Kansas show different seasonal phenologies than those of coastal populations in Texas (Teel et al. 1998, Semtner and Hair 1973). This seasonal variation could mean one population might be a more efficient vector than another.

Price (1977) made the prediction that parasites would occur in small homogenous populations with little gene flow between populations because tick movement is limited by their hosts. Also, variability within a population would be smaller than between populations because of inbreeding. However, Hilburn and Sattler (1986) suggested ticks will have an absence of genetic divergence between local populations over a large geographic area and higher levels of divergence within populations than those suggested by Price (1977). They propose that population size and migration are important factors in determining genetic heterozygosity in ticks.

There have been many studies using genetic markers to look at variability in populations of disease vectors. An example of one of these genetic studies includes a study on *Boophilus,* the vector of *Babesia,* which used isozyme electrophoresis to show that several populations of *Boophilus* have a high genetic similarity (Sattler et al. 1986). Also, a genetic study on *Glossina*, the vector of African trypanosomiasis, proposed a classification of 9 species and subspecies (Gooding 1982), and another study using isozyme electrophoresis on the vector of the bluetongue virus, *Culicoides variipennis*, suggested that some of its subspecies might actually be separate species (Tabachnick 1992).

Any differences between populations could potentially indicate variability in ability to vector diseases. More needs to be known about the differences between

populations of *A. maculatum* to assess associations with host utilization, differences in seasonal activity, and the increasing number of links to diseases. Genetic variation is one mechanism to distinguish between subpopulations and one gene in particular, the mitochondrial 16S rDNA gene, has been shown to have some value in differentiating Gulf Coast tick populations.

Mitochondrial DNA (mtDNA) is a single, duplex, closed circular DNA molecule. In population genetics, the use of mitochondrial DNA is popular over nuclear DNA for many reasons. The genome is small and simple compared to the nuclear genomes of eukaryotic organisms, and mitochondrial DNA has very little or no intergenic sequences, no introns, and rearrangements of sequences are rare. Lastly, mitochondrial DNA is maternally inherited and displays relatively rapid evolution compared to nuclear DNA (Brown 1985). Variation of mtDNA among individuals should be a good indicator of female-mediated gene flow, founder events, and other population level processes according to population genetic theory (Birky et al. 1983). This could also potentially relate to vector competence.

Ribosomes are an important element in protein synthesis and their structures are strictly conserved. The 16S rRNA gene is a small ribosomal subunit and its DNA component, 16S rDNA gene, has been a powerful tool for investigating the evolutionary relationships between organisms. The use of the 16S rDNA gene sequences for classification and identifying different populations within a species is based on the assumption that differences within the sequences depict evolution of the organism (Ueda et al. 1999).

The ideal method to detect specific nucleotide variation is through DNA sequencing, however, this can be an expensive and cumbersome process if a large number of samples are being screened. Fortunately, other methods are available.

A simple method to detect genetic differences is the use of single-strand conformation polymorphism (SSCP) (Orita et al 1989). SSCP is based upon the fact that the electrophoretic mobility of a single-strand DNA molecule in a non-denaturing gel is dependent on both its shape and size. The primary sequence of the single-strand molecule will determine the amount and location of any secondary base pairing. The secondary and tertiary structure of the molecule is then dependent on the length, number, and location of these intra-strand base pairs. If point mutations occur in the molecule that affect the intra-strand interactions then the overall shape of the molecule will change and its mobility on the gel during electrophoresis is altered (Hiss et al. 1994). According to Hayashi (1991), 99% of point mutations in DNA molecules 100-300 base pairs (bp) in length and 89% of point mutations in DNA molecules 300-450 bp in length can be detected by SSCP.

SSCP involves the use of the Polymerase Chain Reaction (PCR) to amplify a small region of the 16S mitochondrial gene, followed by heat denaturization of the DNA amplification product into single strands. The samples are then rapidly plunged onto ice to allow intra-strand complexes to occur so the DNA fragments can fold into 3 dimensional structures according to their primary sequence. The ice will also minimize the renaturization of complementary single strands. The strands then undergo electrophoresis on a nondenaturing polyacrylamide gel at room temperature so the intra-strand complexes are not disturbed and the fragments can separate based on their shape.

Next, the gel is silver stained to detect DNA fragments. Even if two sequences differ by a single nucleotide it is possible that they will fold into different shapes which will affect their mobility on the gel (Hiss et al. 1994).

The overall advantages of using SSCP are that the bands of DNA produced on the gel can be isolated for analysis, nonradioactive labeling is used, no special equipment is needed, and methodologically it is a relatively simple process. There are some disadvantages, however, which include the fact that the gels may be hard to interpret and multiple conditions might be needed to detect all variations. Some of the parameters affecting the sensitivity of SSCP are the gel matrix composition, buffer composition, gel temperature during electrophoresis, and the DNA concentration. A change in any of these can alter the possibility of detecting variation in the DNA fragments (Nataraj et al. 1999).

The 16S rDNA gene is a rapidly evolving gene and has been used in numerous population genetic studies (Simon et al. 1994), including studies of ixodid ticks (Norris et al. 1996). Mixon et al. (2006) used it to look at the population structure and demographic history of *Amblyomma americanum* and found this species to have a high haplotype diversity but a low nucleotide diversity. The study found 29 haplotypes for the 16S rRNA gene sequence out of 703 *A. americanum* individuals studied from 9 different populations in Georgia. About 7 haplotypes were found that had a high frequency in the various populations whereas the remaining haplotypes had low frequencies and were only found in one or two individual specimens.

In previous research on the 16S mitochondrial rDNA gene of Gulf Coast ticks, seven different haplotypes were identified (Williams 2002). This study involved a 1999

collection of *A. maculatum* from cattle in Texas (Refugio County), Georgia (Bulloch County), Oklahoma (Payne County), and Kansas (Osage County). Four of the seven haplotypes were unique to the county from which they came (Table 1). Kansas contained three of the four unique haplotypes while Texas had one. The other three haplotypes were shared among some of the populations. One was shared by Texas, Oklahoma, and Kansas, the second was only shared by Oklahoma and Kansas, and the third was shared between Kansas and Georgia. The study concluded by giving evidence to the possible existence of gene flow between populations.

Resampling these populations can provide evidence as to whether gene flow between populations has taken place in the succeeding eight years and if the same haplotype variation still occurs. Also, sampling ticks from other hosts such as dogs or horses could provide new information about the existence of other haplotypes or possible subpopulations that are host specific. Barker et al. (2004) found that adult Gulf Coast ticks were active and could be found on coyotes in January when they are not found on other hosts such as cattle or white-tailed deer. This suggests that a subpopulation of *A.*

Table 1. Haplotype frequencies for the 16S rDNA gene of *Amblyomma maculatum* in the 1999 collection of ticks from cattle in various counties (Data from Williams 2002)

	Haplotype frequency							
County	A	B	C	D	E	F	G	Number of Haplotypes
Refugio County, TX	0.92	0.08	-	-	-	-	-	2
Payne County, OK	0.72	-	0.28	-	-	-	-	2
Osage County, KS (site 1)	0.08	-	-	0.72	0.16	0.04	-	4
Osage County, KS (site 2)	0.52	-	0.40	-	-	-	0.08	3
Bulloch County, GA	-	-	-	-	-	-	1.00	1

maculatum might exist on coyotes or perhaps on canines more broadly and finding such evidence could provide more insight into the zoonotic cycle of *Hepatozoon americanum*.

The goal of this study is to better characterize the genetic variability of *Amblyomma maculatum* by using an SSCP analysis of a 16S mitochondrial rDNA gene fragment. This information could be vital to progress towards studying *A. maculatum's* vector competence and capacity for pathogen transmission.

OBJECTIVE I: To compare the occurrence and distribution of the 16S mitochondrial rDNA haplotypes of Gulf Coast ticks collected at site-specific locations in Texas and Oklahoma in 1999 with ticks collected from the same locations in 2007. I hypothesize that the frequency and distribution of haplotypes for the 16S mtDNA gene will have changed over the 8 year period.

OBJECTIVE II: To compare 16S rDNA haplotypes among Gulf Coast ticks collected from cattle collected in new geographic areas. I hypothesize the frequency and distribution of haplotypes will show there to be considerable genetic variation of ticks collected from cattle in new geographic areas.

OBJECTIVE III: To compare 16S rDNA haplotypes among Gulf Coast ticks collected from different hosts. I hypothesize the frequency and distribution of haplotypes will show there to be considerable genetic variation of ticks from the different hosts.

CHAPTER II

MATERIALS AND METHODS

Species Collection

The original sites for the 1999 collection were Lake Carl Blackwell Research Station in Oklahoma and Shay Ranch in Texas (Figure 4). These were chosen on the basis that these sites have a long history of established *A. maculatum* populations. In order to compare whether there has been a change in the frequency and distribution of haplotypes for the 16S rDNA gene over time, these sites were resampled by collecting *A. maculatum* from cattle at the Lake Carl Blackwell Research Station in May 2007 and from Shay Ranch in October 2007. The Lake Carl Blackwell Research Station is located in Payne County and is situated in the Cross Timbers ecological area of Oklahoma. The Cross Timbers area ranges from open savannah to dense brush, mostly post oak (*Quercus stellata*) and blackjack oak (*Quercus marilandica*). The principal grasses include little bluestem (*Schizachyrium scoparium* var. *frequens*), big bluestem (*Andropogon gerardi*), Indian grass (*Sorghastrum nutans*), switchgrass (*Panicum virgatum*), and Canada wild-rye (*Elymus canadensis*). Shay Ranch is located in Refugio County and is situated in the Gulf Prairies ecological area of Texas. The vegetation of the Gulf Prairies is generally tall grasses such as big bluestem, seacoast bluestem (Schizachyrium *scoparium* var. *littoralis*), and Indian grass. The area also contains trees and brush such as mesquite (*Prosopis glandulosa*), oaks (*Quercus* spp.), prickly pear (*Opuntia* spp.), and acacias.

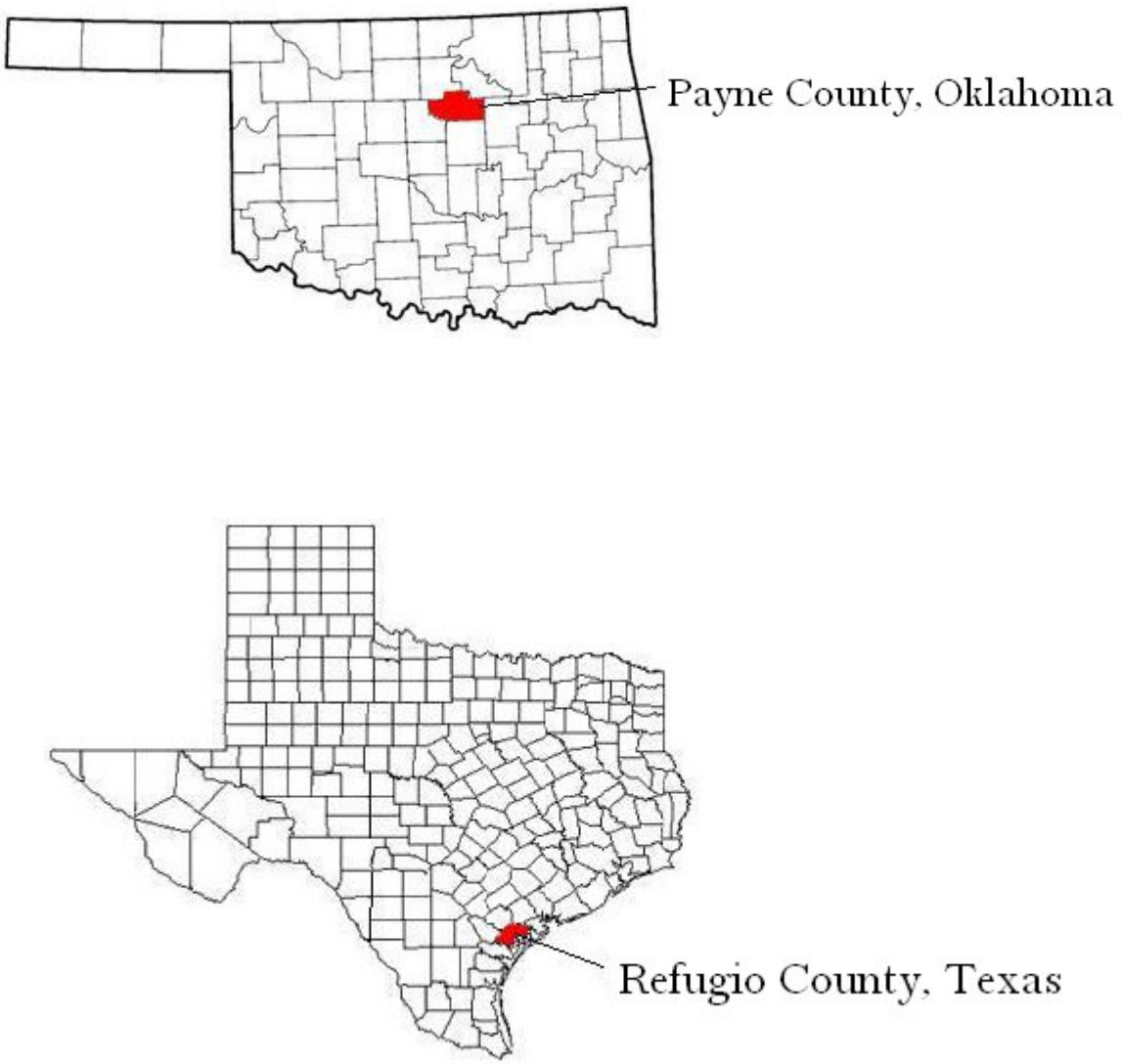

Fig. 4. County locations of the *Amblyomma maculatum* collections in 1999 and 2007 for Lake Carl Blackwell Research Station in Oklahoma and Shay Ranch in Texas.

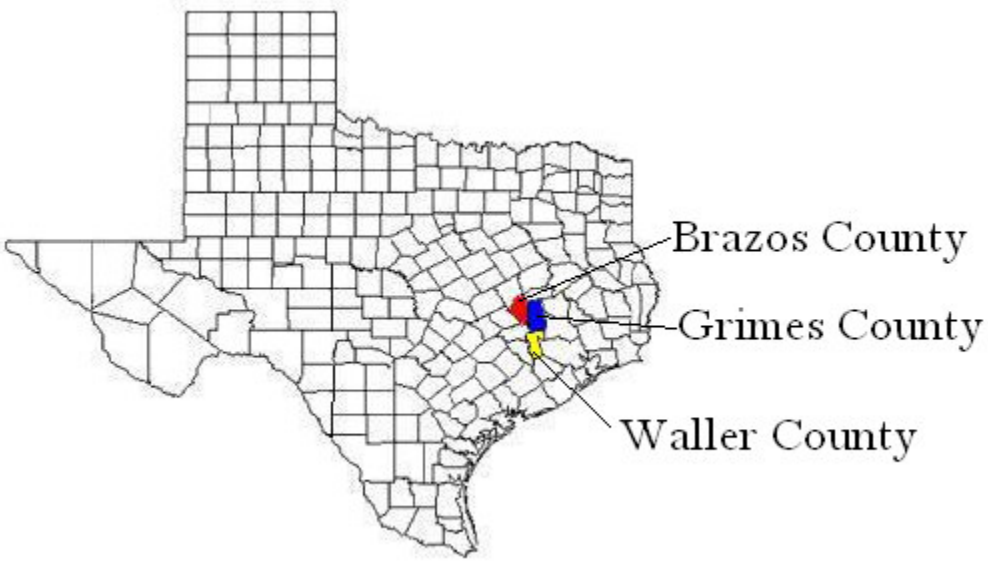

Fig. 5. County locations for the 2007 *Amblyomma maculatum* collections include Brazos County for the Texas A&M Riverside Campus and Brazos, Grimes, and Waller County for the original location of the canines brought into the Brazos Animal Shelter.

Ticks were also sampled from cattle, on June 27, 2007, and horses, on June 29, 2007, at the Texas A&M University Riverside Campus, located in Brazos County, and from canines brought into the Brazos Animal Shelter, from July 26 to September 28, 2007, in order to compare the frequency and distribution of haplotypes from different hosts and new geographic areas. The canines were originally from Brazos County, TX, Grimes County, TX, and Waller County, TX (Figure 5). These sites are all located in the Post Oak Savannah of east central Texas. The trees of the Post Oak Savannah are mostly post oak and blackjack oak and the grass is generally comprised of little bluestem, Indian grass, and switchgrass (Correll and Johnston 1970). Also, one tick was collected from a human working in a horse pasture in Brazos County.

Two female ticks were also collected from cattle in Callahan, Florida (Nassau County), one male tick was collected in Ocala, FL (Marion County), and two male ticks were collected in Gainesville, FL (Alachua County) (Figure 6).

Williams (2002) only processed 25 ticks for each of the collection locations in order to look for haplotypes of the 16S rDNA gene. With a larger sample size it might be more likely that different or rare haplotypes can be found and so every *A. maculatum* tick that was collected was also processed for this present study. The ticks collected from cattle and horses were obtained by physical inspection of individual animals. Ticks were either stored alive in vials, labeled for location, date and host, and placed in cool moist conditions for transfer to the laboratory or they were placed in 80% ethanol for shipping. All *A. maculatum* ticks were collected from canines as individual dogs were brought into the Brazos Animal Shelter. The total number of ticks collected from Payne County,

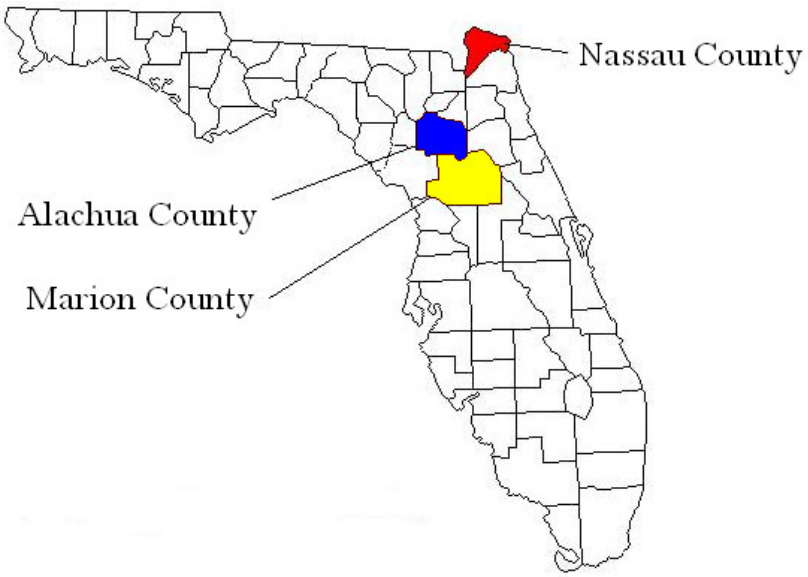

Fig. 6. Florida county locations of collected *Amblyomma maculatum* specimens.

Oklahoma was 46, in Refugio County, Texas there were 243 collected, in Brazos County, Texas 23 ticks were collected from horses, 57 from cattle, and 28 from dogs.

DNA Extraction

DNA was extracted using the following modifications to the Black IV and DuTeau (1997) procedure for the Texas and Florida tick collections: 1) individual live ticks (either male or female) were placed on a sterile Petri dish and all 8 legs were removed at the level of the coxae using a sterile razor blade, 2) the body of the tick was then place in a vial and labeled based on host, location, and was assigned a number so that individuals could be catalogued and the vial was stored at -70 ºC, 3) the legs were placed in a 1.5mL microcentrifuge tube, snap frozen in liquid nitrogen, and immediately ground for twenty seconds using a KONTES® disposable pestle, 4) 25 µL of Lifton Grinding Buffer (25µL of 0.1 M Tris buffer pH 9.0, 0.2 M sucrose, 0.05 M ethylamine-diaminetetraacetate (EDTA), and 0.5% sodium dodecyl sulfate (SDS)) was added to the pellet and the legs were ground for 90 seconds. 5) 25 µL of Lifton Grinding Buffer was then used to wash the pestle, 6) the DNA was resuspended in 25µL of TE (0.01 M Tris-HCl pH 8.0 and 1mM EDTA) or ddH_20 and stored at -20ºC.

The following modifications to the Black IV and DuTeau (1997) procedure were followed for the entire bodies of live ticks from the Oklahoma collection: 1)individual ticks were placed on a sterile Petri dish and cut into 8 or 9 small pieces using a sterile blade and placed into a 1.5mL microcentrifuge tube, 2) Phosphate Buffered Saline solution was added to the microcentrifuge tube containing the tick pieces, 3) a Sonic dismembrator (Fisher Scientific, Pittsburgh, Pennsylvania) was used to disrupt tissues and homogenize the contents of the microcentrifuge tube 4) the tube was spin-collected

and the supernatant removed, 5) Lifton Grinding Buffer (25µL of 0.1 M Tris buffer pH 9.0, 0.2 M sucrose, 0.05 M ethylamine- diaminetetraacetate (EDTA), and 0.5% sodium dodecyl sulfate (SDS)) was then added to the pellet and the homogenate was incubated at 65ºC for 30 minutes, 7) the DNA was resuspended in 50µL of TE (0.01 M Tris-HCl pH 8.0 and 1mM EDTA) or ddH_20 and stored at -20ºC.

Selection of Primers

Primers selected for the 303 bp region of the 16S mitochondrial rDNA gene were chosen based upon multiple tick gene sequences for the region available through GenBank (Accession Number: L34318). The same primers were used by Williams (2002) and were as follows:

Forward 5'- TAA GGA CAA GAA GAC CCT AAG A-3'

Reverse 5'- GTC TGA ACT CAG ATC AAG TAG G-3'

Polymerase Chain Reaction

A MasterCycler personal (Eppendorf, Hamburg, Germany) was used to amplify the 303 bp region of the 16S mitochondrial rDNA gene from the extracted DNA. The PCR reaction solution contained 13µL of a master mix (0.25µL forward primer, 0.25µL reverse primer, 0.5µL 10mM dNTPs, 1.5µL 25mM $MgCl_2$, 1.5µL 10X PCR Buffer ($MgCl_2$ free), 0.25µL Ampli*Taq* Gold DNA polymerase, and 8.75µL ddH_2O), to which 2µL of extracted DNA template was added.

A preincubation period was used for 5 minutes at 95ºC. This was followed by an incubation period at 94 ºC for 30 seconds, an annealing phase of 58ºC for 30 seconds, and an extension phase of 72ºC for 30 seconds. The incubation, annealing, and extension

periods were repeated 39 times and the amplification ended with a 5 minute final extension phase at 72ºC.

The amplified DNA was visualized by running 7µL of the PCR reaction on a 2% agarose gel containing ethidium bromide and by using an ultra-violet transilluminator to image the gel.

Single Strand Conformation Polymorphism (SSCP) Analysis

Polyacrylamide TBE Gel Electrophoresis (PAGE) was performed by using the protocol for the XCell *Surelock* Mini-Cell system (Invitrogen, Carlsbad, California). For this, 5 µL of the PCR product was denatured at 98ºC for 5 minutes and then cooled on ice for 2 minutes. The PCR product was then mixed with 2 µL of TBE sample buffer and loaded onto a 1 mm gel. The gel was run at room temperature with constant amperage of 15 mA until the dye reached the bottom of the gel.

Silver staining was conducted by using the SilverXpress Silver Staining Kit (Invitrogen) in a 1.5 L glass container. Any unique haplotypes were identified and were run on a polyacrylamide TBE gel with the 7 previously identified haplotypes found by Williams (2002) and confirmed by DNA sequencing. The patterns on the gel produced by the haplotypes found from the ticks collected in 2007 were compared to the patterns of the 7 haplotypes found in the 1999 collection to determine if the previous haplotypes were still present within the Gulf Coast tick populations and if novel haplotypes had emerged.

It should be noted that at times the SSCP haplotype patterns on the polyacrylamide gel were difficult to interpret because the banding patterns of the DNA fragments for the different haplotypes have small variations. In order to determine the

haplotype for DNA fragments that had ambiguous banding patterns, the individual ticks linked to the ambiguous banding patterns had their extracted DNA reamplified using PCR and the PCR product was run on another polyacrylamide TBE gel for comparison.

Analysis

The statistical analysis program SAS WIN 9.1.3 (Statistical Analysis System version 9.1.3 Cary, N.C.) was used to perform a chi square homogeneity of proportions test with an exact test to compare haplotype frequencies found in the Texas and Oklahoma counties. This provided evidence as to whether the haplotype frequencies have remained the same over the past eight years, if haplotype frequencies are the same across all geographic locations, and if haplotype frequencies on different hosts are the same.

CHAPTER III

RESULTS

Single-strand conformation polymorphism was used to detect haplotypes of the 16S rDNA mitochondrial gene of *Amblyomma maculatum* from samples collected in 2007 from Payne County, Oklahoma, and Refugio and Brazos County, Texas. A summary of the SSCP patterns for the haplotypes found in the samples are shown in Figure 7. Only 5 haplotypes (A, B, C, D, G) were recovered out of the 7 haplotypes (A, B, C, D, E, F, G) found in 1999 by Williams (2002). It was believed that with larger sample sizes novel haplotypes would be identified although this did not occur.

Payne County, Oklahoma had the most haplotypes while Florida had the least (Table 2). Haplotype D was found in all locations and from all hosts that were sampled and it also had the highest frequency out of all the haplotypes. *Amblyomma maculatum* found in Marion and Alachua Counties, Florida had haplotype D while the two ticks

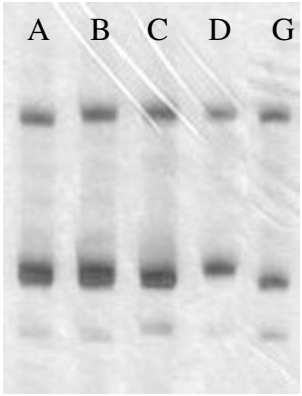

Fig. 7. Stained polyacrylamide gel showing single strand conformation polymorphism (SSCP) for the 16S mitochondrial rDNA gene haplotypes of *Amblyomma maculatum*. Haplotypes are designated A, B, C, D, and G.

Table 2. Summary of *Amblyomma maculatum* haplotype frequencies for the 16S mitochondrial gene identified for each of the hosts and counties sampled from Oklahoma, Texas, and Florida in 2007

				2007 Haplotype Frequency							
State	County	Host	# Ticks Sampled	A	B	C	D	E	F	G	# of Haplotypes
Oklahoma	Payne	Cattle	46	0.196	0.152	0.109	0.543				4
Texas	Brazos	Horse	23	0.174	0.260		0.565				3
Texas	Brazos	Human	1				1.000				1
Texas	Brazos, Grimes, Waller	Dog	28	0.214	0.107		0.679				3
Texas	Brazos	Cattle	57	0.228	0.175		0.596				3
Texas	Refugio	Cattle	243	0.091	0.222		0.687				3
Florida	Nassau	Cattle	2				0.500			0.500	2
Florida	Marion	Cattle	1				1.000				1
Florida	Alachua	Cattle	2				1.000				1

found in Nassau County had haplotype D and G. This was the only instance when haplotype G was found. In the 1999 collection haplotype G was found in Bulloch County, Georgia. The fact that G appeared in Nassau County is not surprising because of its close proximity to Georgia. The solitary tick found on a human in Brazos County was of haplotype D.

Comparison of the 1999 and 2007 Oklahoma and Texas Collections

Payne County, Oklahoma

The results from the SSCP for the analysis of the 16S mitochondrial rDNA gene from the 2007 Oklahoma collection were compared to the 1999 collection by Williams (2002). Table 3 shows there were 2 haplotypes, A and C, found in 1999 but 4 haplotypes, A, B, C, and D, found in 2007. Not only was there an increase in the number, but there also seemed to be a change in distribution of the haplotype frequencies. In 1999, haplotype A comprised 72% of the collected Gulf Coast ticks, where as in 2007 it only made up 19.6%. Haplotype C made up 28% of the Payne County collection in 1999 but in 2007 it was only 10.9%. It is interesting to note that haplotype D, which was not present in 1999, was detected in 54.3% of the ticks sampled from the 2007 Oklahoma population.

Table 3. *Amblyomma maculatum* haplotype counts for the 16S mitochondrial rDNA gene for Payne County, Oklahoma in 1999 (Williams 2002) and 2007

Year	Total Specimens	Haplotype			
		A	B	C	D
1999	25	18 (72.0%)	0 (0%)	7 (28.0%)	0 (0%)
2007	46	9 (19.6%)	7 (15.2%)	5 (10.9%)	25 (54.3%)

A chi-squared homogeneity of proportions with an exact test was conducted to statistically determine if there was a change in the distribution of haplotype frequencies. This resulted in a p-value of 5.15×10^{-8}, so with an $\alpha = 0.05$ the Oklahoma population sampled in 2007 can be said to have had a different composition of haplotype proportions than those found in the 1999 sample.

Refugio County, Texas

Haplotype identification and distribution of the 16S rDNA gene for the 2007 Refugio County, TX collection was compared to the results from the 1999 collection (Williams 2002). This yielded results similar to those of the Oklahoma comparisons. Table 4 shows that in 1999 there were only two haplotypes, A and B, however in 2007 haplotype D was also found. Similar to the Oklahoma data, haplotype A had a considerable drop in frequency from 1999 were it was in 92% of the population to only 9% in 2007, haplotype B increased in frequency from 8% in 1999 to 22.2% in 2007, but the most interesting change involves haplotype D. As with Oklahoma, haplotype D was not present in Texas in 1999 yet within an eight year period it is now found in 68.7% of the population, so it appears obvious that the 1999 and 2007 populations are different.

Table 4. *Amblyomma maculatum* haplotype counts for the 16S mitochondrial rDNA gene for Refugio County, Texas in 1999 (Williams 2002) and 2007

Year	Total Specimens	Haplotype		
		A	B	D
1999	25	23 (92.0%)	2 (8%)	0 (0%)
2007	243	22 (9.1%)	54 (22.2%)	167 (68.7%)

A statistical analysis, using a chi-squared homogeneity of proportions with an exact test, with an $\alpha = 0.05$ confirms this with a p-value of 7.004×10^{-20}.

Comparison of the 16S rDNA Gene of Gulf Coast Ticks Found on Cattle in 2007

The haplotypes for the 16S rDNA gene found in the ticks collected from cattle were compared to determine whether the Gulf Coast ticks had the same haplotype frequencies in Refugio County, Brazos County, and Payne County in 2007. The haplotype counts for each of the counties are displayed in Table 5. A chi-squared homogeneity of proportions and an exact test was conducted for Brazos and Refugio, Brazos and Payne, and Refugio and Payne counties. The resulting p-values for these test showed that with an $\alpha = 0.05$ Brazos and Refugio counties ($p=0.0135$) and Refugio and Payne counties ($p=5.723 \times 10^{-6}$) do not have the same haplotype frequencies in their populations, while Brazos and Payne counties ($p=0.0879$) do have the same frequency distributions.

Table 5. Haplotype counts for the 16S mitochondrial rDNA gene from *Amblyomma maculatum* collected from cattle in different counties of Texas in 2007

County	Total Specimens	Haplotype			
		A	B	C	D
Brazos	57	13 (22.8%)	10 (17.5%)	0 (0%)	34 (59.7%)
Refugio	243	22 (9.1%)	54 (22.2%)	0 (0%)	167 (68.7%)
Payne	46	9 (19.6%)	7 (15.2%)	5 (10.9%)	25 (54.3%)

Haplotype C was present in Payne County but absent in Brazos County. This haplotype was also not found in any of the other tick collections from dogs or horses within Brazos County and the surrounding areas. It seems these two populations are

different in terms of the number of haplotypes for the 16S rDNA gene, even though the haplotype frequencies appear to be similar.

Comparison of the Gulf Coast Ticks from Various Hosts

Gulf Coast ticks were collected from cattle, horses, and dogs from Brazos County and surrounding areas. An SSCP analysis was conducted on the 16S rDNA gene found from these ticks and the haplotypes of this gene were then compared. Table 6 shows that all three hosts had haplotypes A, B, and D and no others. Conducting a chi-squared homogeneity of proportions and an exact test resulted in a p-value of 0.6991, and with an $\alpha = 0.05$ it appears the Gulf Coast ticks found on different hosts have the same haplotype frequencies.

Table 6. Haplotype counts for the 16S mitochondrial rDNA gene from *Amblyomma maculatum* collected from cattle, dogs, and horses in Brazos, Waller, and Grimes County, Texas in 2007

Host	Total Specimens	Haplotype		
		A	B	D
Cattle	57	13 (22.8%)	10 (17.5%)	34 (59.7%)
Dog	28	6 (21.4%)	3 (10.7%)	19 (67.9%)
Horse	23	4 (17.4%)	6 (26.1%)	13 (56.5%)

CHAPTER IV

DISCUSSION

Comparison of the 1999 and 2007 Oklahoma and Texas Collections

The mitochondrial haplotype frequencies for the 16S rDNA gene from the Refugio County, Texas and Payne County, Oklahoma populations shows an obvious shift from a dominant haplotype A in 1999 to a dominant haplotype D in 2007 (Tables 3 & 4). In 1999, haplotype D was only seen in Osage County, Kansas, although it had a high frequency of 0.72 (Table 1) (Williams 2002). There are two possible explanations as to why haplotypes B and D are now seen in Oklahoma and haplotype D is now seen in Texas; one being the dispersal and transfer of *A. maculatum* by hosts and the other being an extremely low preexisting presence within these states.

The movement of *A. maculatum* over large expansive areas is dependent on the hosts they parasitize. The Gulf Coast tick is known to utilize a wide variety of mammals and birds. In fact, *A. maculatum* larva and nymphs are known to feed upon an array of ground dwelling birds such as the meadow lark and the grasshopper sparrow, which are distributed throughout the Great Plains and are known to winter in its southern regions (Bishopp and Trembley 1945). The flyway for many of these birds extends from Kansas to the Gulf Coast of Texas, so haplotypes B and D could have been dispersed through Oklahoma and Texas by these birds. Other wildlife *A. maculatum* is known to parasitize in both Oklahoma and Texas include white-tailed deer, coyotes, and hogs (Hooker et al. 1912, Semtner and Hair 1973), all of which have sizeable ranges and could help

facilitate the movement and distribution of the Gulf Coast tick. It would be useful to have models of rate dispersal for ticks however there is a lack of research in this area so it is difficult to fully understand how tick populations spread and interact with one another.

Species that have high dispersal ability exhibit less population differentiation than those with a more limited means of dispersal, because populations with dispersal capabilities have more opportunities for gene flow (Avise et al. 1987). The fact *A. maculatum* parasitizes a variety of hosts with large ranges and flyways and that haplotypes are shared between counties supports this view.

The sample sizes of both the 1999 collections for Oklahoma and Texas were smaller than those from 2007. In 1999, the sample size was only 25 ticks for both of the collection sites. Conducting an examination of the probability that a specific haplotype would be detected in an individual tick shows that haplotypes B and D could have existed in these populations in very low numbers. For example, if P is the probability that a Gulf Coast tick is haplotype D, P=Pr[Tick is Haplotype D], then the probability of not seeing any haplotype D within a sample of size n is $(1-P)^n$. Therefore, the probability of seeing at least one individual with haplotype D within a sample of size n is $1-(1-P)^n$. Table 7 shows that if a particular haplotype is in 1% of the population then there would only be a probability of 0.222 that at least one individual will have this haplotype in a sample of size of 25. The probability increases to 0.723 if the haplotype is in 5% of the population. However, if haplotype D was in 54.3% or 68.7% of the population (Tables 3 and 4) the probability of seeing at least one individual with this

haplotype would be almost be guaranteed (probability of 1.000). This provides some evidence that there has been a dramatic change in frequencies over the past eight years. If haplotype D had the same frequency in 1999 and 2007 then at least one tick with this haplotype would have been present in the 1999 sample population. Also, one other possibility is that the method of selecting the 25 ticks in 1999 was not a random sample over a wide enough geographic range. A combination of both the dispersal of ticks due to host movement and the haplotypes' presence from previous years could have contributed to their existence in the 2007 collection of ticks.

Table 7. Probability that at least one specific 16S mitochondrial rDNA haplotype will be seen in an individual *Amblyomma maculatum* with a sample size of 25 given different proportions in the population

Proportion of a specific haplotype in a population P	Probability of not seeing the specific haplotype in a sample size of 25 $(1-P)^{25}$	Probability of seeing at least one individual with the specific haplotype $1-(1-P)^{25}$
0.01	0.777821	0.222
0.05	0.27739	0.723
0.1	0.07179	0.928
0.2	0.003778	0.996
0.3	0.000134	0.99987
0.4	2.84E-06	0.999997
0.5	2.98E-08	1.000
0.6	1.13E-10	1.000
0.7	8.47E-14	1.000

These two factors alone do not explain how haplotype D could have come to dominate over the others in as little as 8 years. There are however several possible explanations as to why there has been such a drastic change in dominance. Females

possessing haplotype D might have a much higher fecundity than others. If female ticks with haplotype D had recently entered Texas and Oklahoma in 1999 and were only present in low numbers but they had a higher fecundity rate, then it could be a possible that they would dominate and out compete other haplotypes of *A. maculatum*. Testing this hypothesis could be conducted by rearing *A. maculatum* until the adult stage and then applying them to cattle and letting them feed. Afterwards, records could be kept on the mass of females at engorgement, mass of eggs at completion of oviposition, and the percentage of eggs hatching to larvae; then the haplotypes of the females could be determined and any correlation between fecundity and haplotype could be discerned.

Another cause that might explain the data is that haplotype frequencies could fluctuate yearly or every few years, especially in terms of ticks actively seeking hosts or being found on hosts. There has been no research testing whether haplotype frequencies in any tick species remains constant year to year. Any fluctuations could be due to environmental conditions affecting the fecundity of the ticks of the various haplotypes in differing manners and also affecting their overall fitness. Also, it is possible that ticks with specific haplotypes will actively seek out hosts in response to specific environmental conditions. It is known that ticks can survive long periods without feeding, and thus they might only choose to seek out a host under preferred environmental conditions such as a range of temperatures or humidity levels. This could be tested by sampling Gulf Coast ticks year to year over several years in a field population while also sampling a laboratory colony, which originally consists of the same haplotypes as the field site. The ticks of the field study would be stressed under

the naturally changing environmental conditions from each year while the laboratory colony conditions would be kept the same. Another way this could be tested is by creating a lab colony consisting of the different haplotypes, varying the environmental conditions, and observing any changes. This might show whether the environment causes any fluctuation in haplotype frequencies from year to year due to altering fecundity, changing the tick's overall fitness, or by influencing the tick's behavior to actively seek out a host.

Another possible explanation for why there has been a large emergence of haplotype D is that it might be more resistant to acaricides. Most pesticide treatment of cattle generally focuses on hornflies and any change of pesticides is for hornfly resistance. Over the past 10 years there has not been any large scale change in the class of acaricides used on cattle and information on any changes at the tick collection sites was unattainable. Also, it appears that cattle ranchers only use minimal treatment and prevention in tick control. For instance, cattle ranchers generally tag cattle with pesticide-impregnated ear tags in the spring for fly control while sorting the herd (personal correspondence). However, adult ticks do not have peak levels of activity in Texas until September. Pesticide-impregnated ear tags are only good a few months at best and so by the time adult activity is at its peak the ear tags are nearly ineffective. While collecting ticks in October, Gulf Coast ticks can often be seen feeding right under the pesticide-impregnated ear tag (personal observation). This improper use of pesticides, when it comes to tick control, could allow for a resistance to build in the

population and could possibly explain the large emergence of haplotype D if it provides some level of resistance.

Amblyomma maculatum utilizes a wide variety of hosts, including cattle, so even though resistance is a possible scenario it is an unlikely cause of the dominance of haplotype D. In order for a large emergence of haplotype D due to pesticide resistance to result, it would mean that cattle would have an extremely large influence on the Gulf Coast tick population. There is no denying that cattle and any acaricide treatments used on them have some effect on the ticks, however feral hogs, white-tail deer, and a number of other species are known to carry Gulf Coast ticks, and have no tick control plans in use, suggesting that pesticides used just on cattle would not completely change the haplotype frequencies of the *A. maculatum* population. However it still somehow could be affecting the population. In order to test whether haplotype D is more resistant, a bioassay could be conducted using the pesticides currently in use and Gulf Coast ticks of a known haplotype.

Another explanation for the increase in haplotype D could be due to a higher tolerance for drought. Gulf Coast ticks are sensitive to desiccation at all stages in their life cycle (Hixson 1940, Hair et al. 1975, Fleetwood 1985), however any drought conditions during the immature stages will influence which ticks will make it to the adult stage and will impact what adults are found on hosts. If haplotype D has a higher tolerance for dry conditions then it might be able to increase in frequency during dry years.

In 1998, drought conditions did occur in Texas and Oklahoma yet haplotype D was not observed. It is possible that haplotype D might not have been present yet or had only just reached these areas and were only in low numbers. Data collected from the U.S. Forage Condition Weather Data System (online database) shows the monthly precipitation for each year from 1998 through 2007 for Payne and Refugio County (Figures 8 and 9).

The U.S. Drought Monitor observes and tracks drought using multiple indices and categorizes it into abnormally dry, drought-moderate, drought-severe, drought-extreme, and drought-exceptional. According to the U.S. Drought Monitor and the information obtained from the U.S. Forage Condition Weather Data System, from 2000 to 2007 much of Texas and Oklahoma were experiencing either abnormally dry or even drought conditions. For instance, Payne County, OK experienced dry or drought conditions from the end of 2001 to mid-2002, from May 2003 until September 2003, and in April 2005 through June 2005. The most serious drought occurred from October 2005 through April 2007, so throughout the entire year of 2006 Oklahoma was experiencing conditions ranging from abnormally dry to extreme drought. Refugio County, TX also experienced dry or drought conditions from January through May 2000, July through November 2000, June through August 2001, March through July 2002, April through July 2003, and November 2006 through January 2007. One of its most serious droughts, since 2000, occurred from June 2005 through July 2006 when it experienced conditions that ranged from abnormally dry to extreme and even exceptional drought.

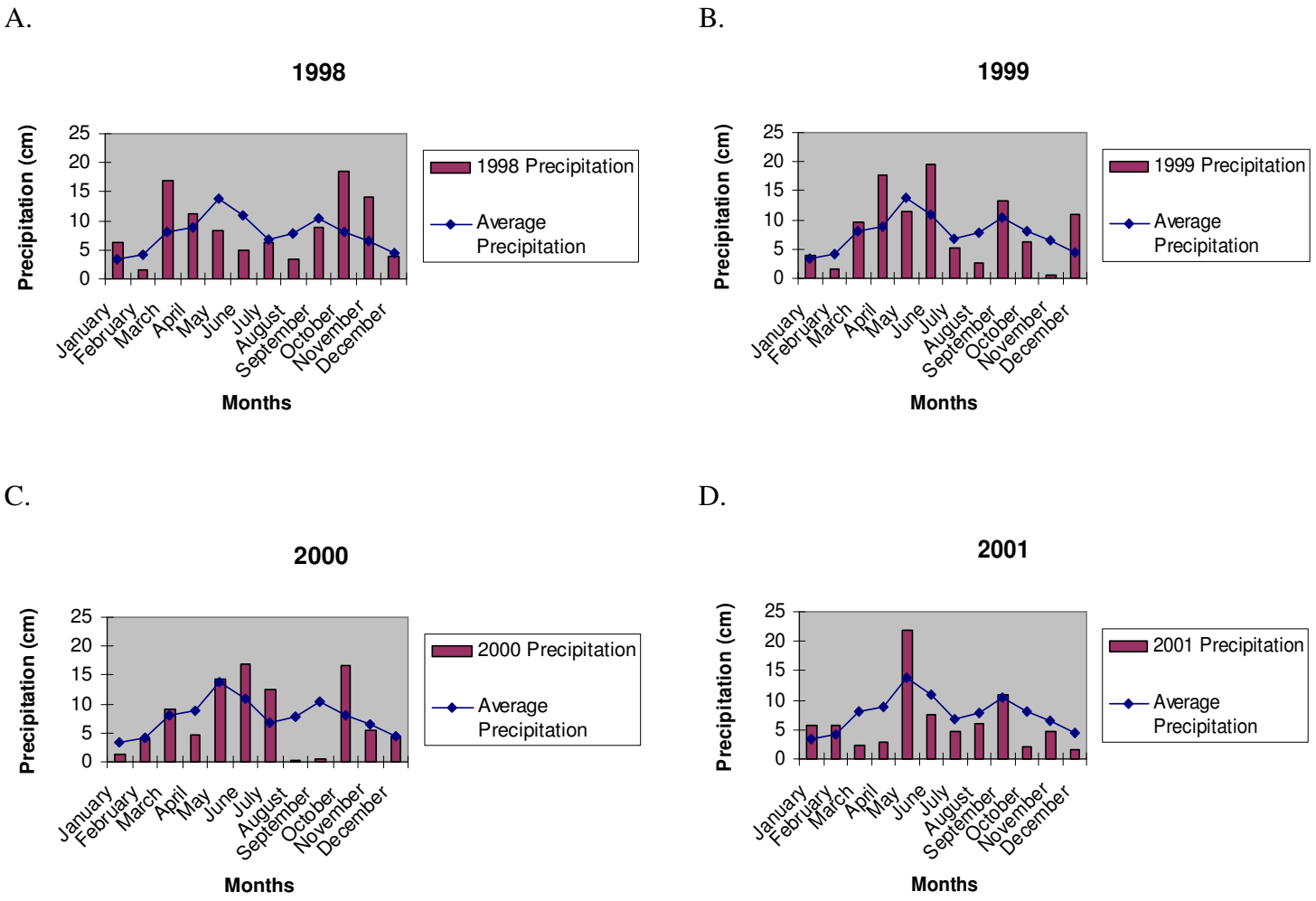

Fig. 8. The monthly precipitation for Payne County, OK in the years 1998 through 2007.

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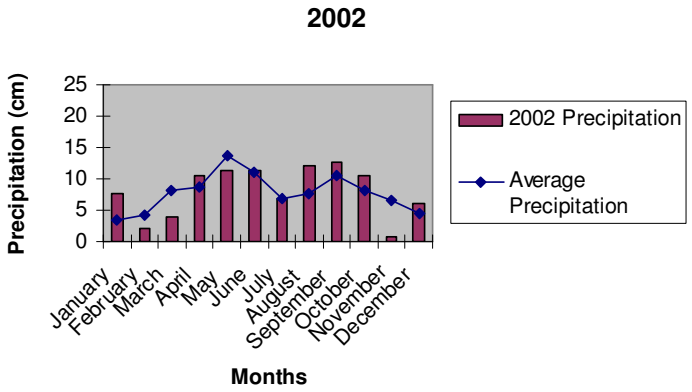

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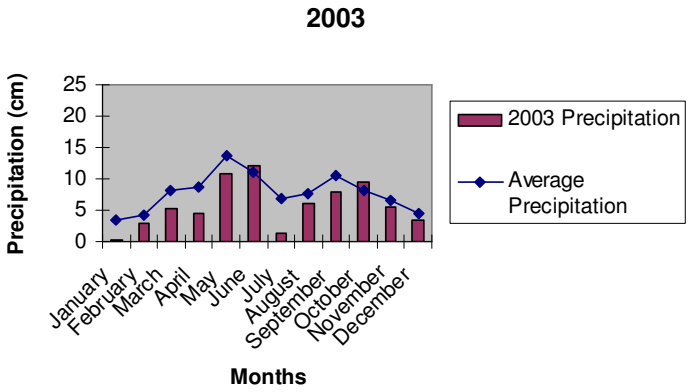

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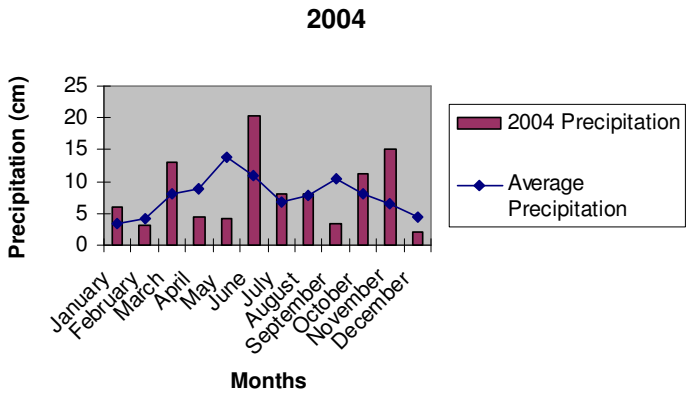

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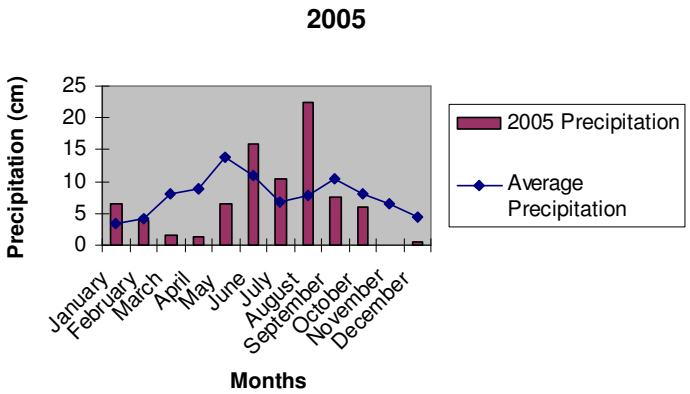

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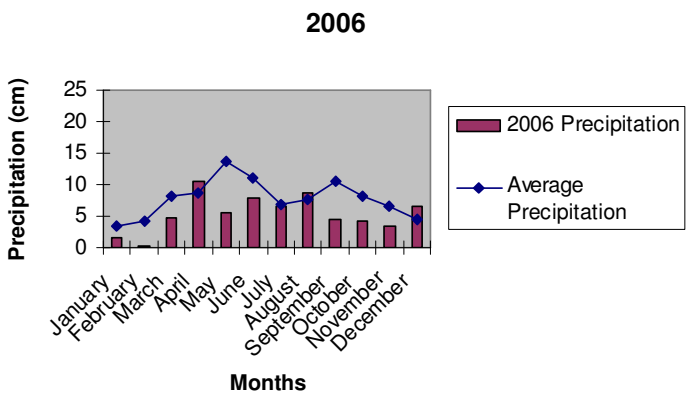

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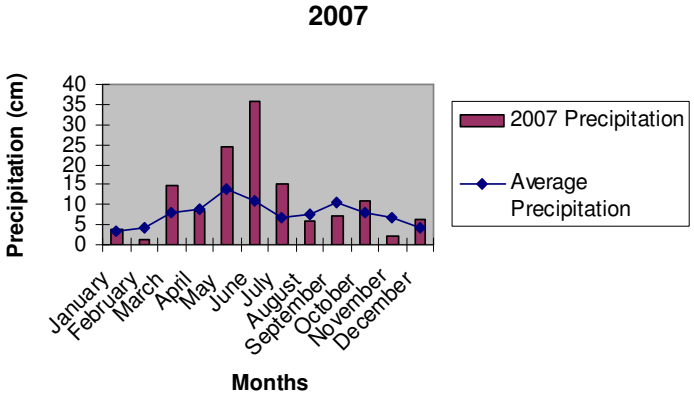

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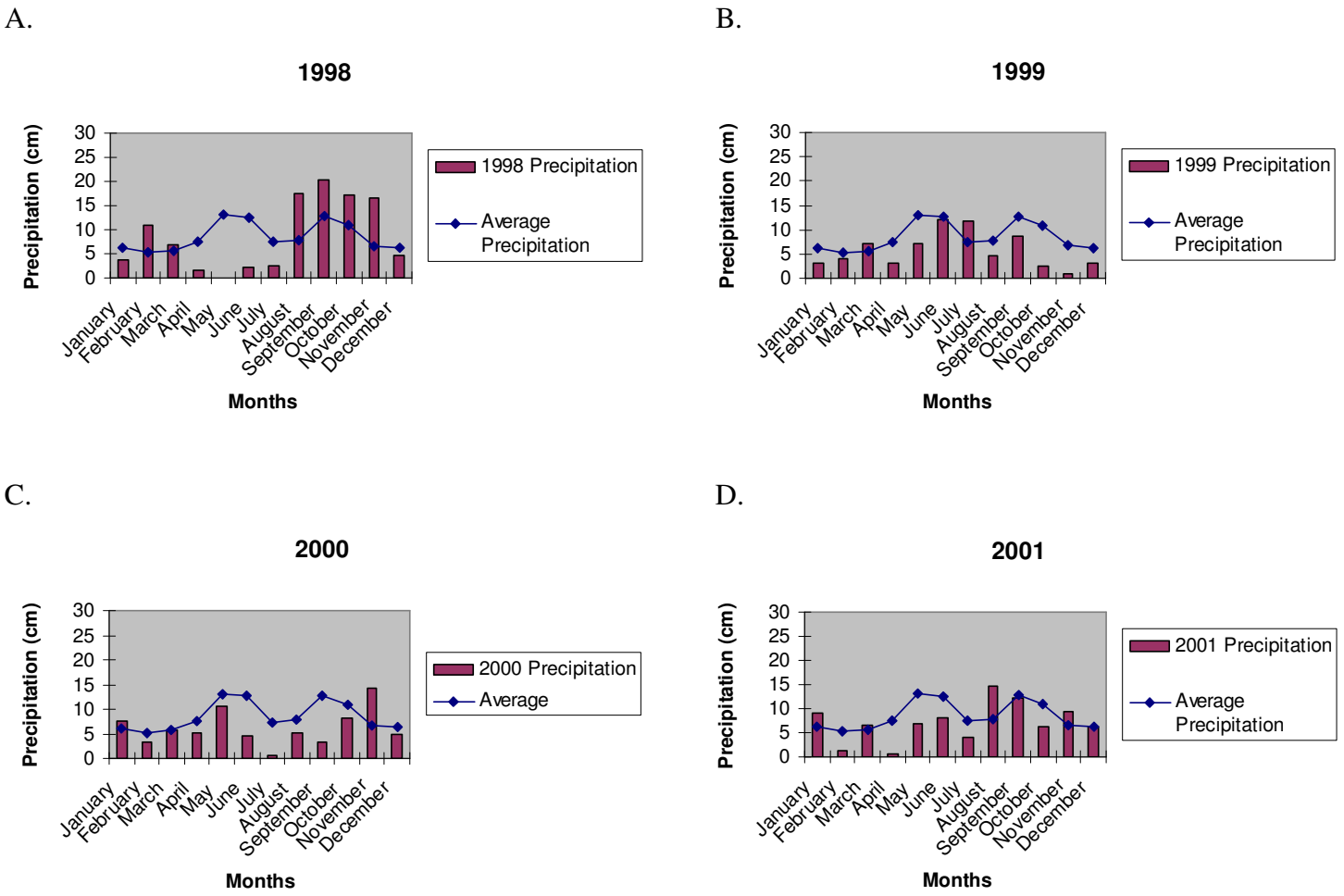

Fig. 9. The monthly precipitation for Refugio County, TX in the years 1998 through 2007.

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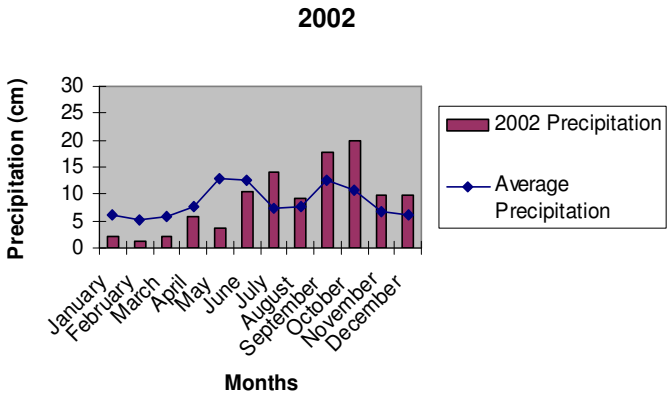

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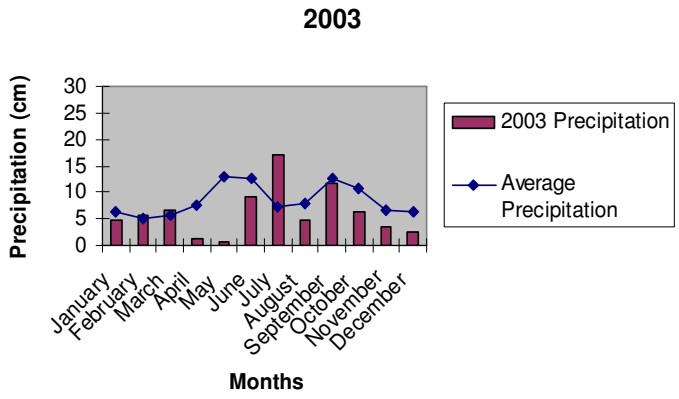

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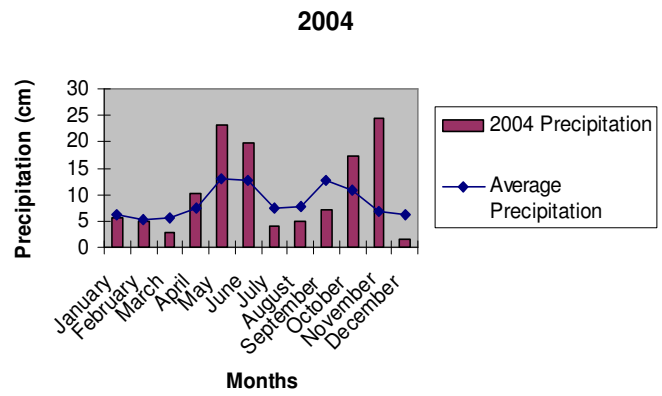

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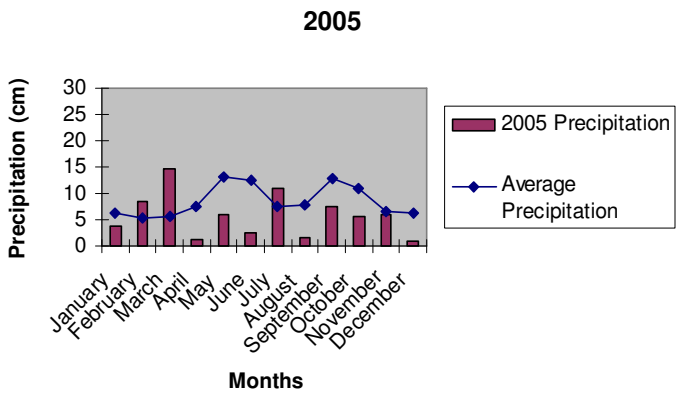
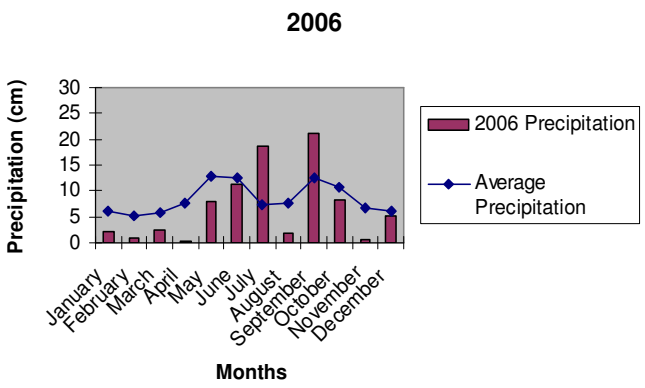


Fig. 9. Continued

I.



J.

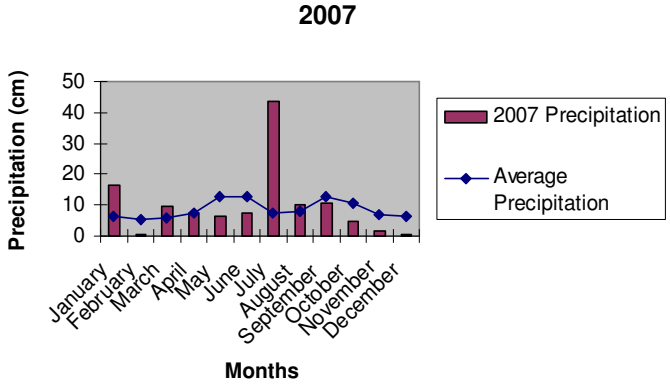


Fig. 9. Continued

Drought conditions in both Texas and Oklahoma seem to occur every year however extreme and exceptional drought conditions occurred in Payne and Refugio County in 2006 and some drought conditions continued on into 2007. This information along with the haplotype frequency change for haplotype D might suggest that it has a higher tolerance for drought than others. If haplotype D had arrived in the Refugio and Payne County populations after 1999 or just arrived in these sites in low numbers in 1999, and if this ticks with this haplotype are more resistant to dry conditions, then the following years would have allowed ticks with this haplotype to start increasing in frequency and could have lead to the frequencies seen today. The other haplotypes that might be associated with a smaller tolerance to dryness could have succumbed to desiccation at the immature or adult stages and not produced any offspring, or produced significantly fewer offspring.

Future work on the drought resistance of the haplotypes could be conducted in two ways. The first would be a long term field study in which tick collection sites would be sampled year to year tracking the haplotype frequencies along with weather conditions. The second could be through lab experiments in which stress on the ticks would be induced by simulating a variety of environmental conditions. This could be done by rearing *A. maculatum* females of known haplotypes and using their offspring at various stages to test their integument's permeability to water loss following the procedure used in Lees (1946) or Needham and Teel (1986). Also, the eggs of the females would also need to be tested which could be done by using the same protocol

Sutherst and Bourne (2006) used to measure the effect of desiccation on eggs and emerging larva of *Rhipicephalus microplus*.

In the future, samples should be obtained from the original Kansas site where haplotype D was first identified (Osage County site 1) by Williams (2002). It would be interesting to see if the haplotype frequencies there are still the same. Also, it would be pertinent to test the other Kansas site, originally tested by Williams (2002), where haplotype D was not found (Osage County site 2). Since haplotype D is now seen in Texas and Oklahoma, it is perhaps likely that haplotype D would be present at site 2 seeing its close proximity to site 1. It also should be noted that the changes in haplotype frequencies are not likely to be related to the function of the 16S rDNA gene. It is more likely that the changes in frequencies are related to other traits while the gene is just a marker.

Comparison of *A. maculatum* on Cattle

The statistical tests conducted for the haplotype frequencies for *A. maculatum* found on cattle in 2007 showed that Refugio County had different haplotype frequencies, and therefore a unique population structure in terms of the 16S rDNA gene, than the other counties. Brazos County and Payne County appear to have similar frequencies, however haplotype C was not found on any of the cattle in Brazos County nor on any of the dogs and horses. These two populations have the same 16S rDNA population structure even though Brazos County does not have all of the haplotypes found in Payne County.

Host Comparison

Barker et al (2004) found a seasonal occurrence of Gulf Coast ticks on various mammalian hosts. In Oklahoma, they found adults ticks in January on coyotes and did not find any other adults on other hosts during this month. In February, they found adult ticks mainly on cattle, in April through June they found the adult ticks on a variety of mammals, and in July and August they only found them on white-tailed deer. This suggested that *A. maculatum* possibly had subpopulations that only feed on certain hosts or that there is a host preference during a specific time of year.

The comparison of haplotype frequencies for the 16S rDNA gene of *A. maculatum* found on cattle, dogs, and horses showed no differences, so at this point, the data does not show whether Gulf Coast ticks with specific haplotypes have any host preferences. This is not altogether surprising. When *A. maculatum* is actively seeking out a host it would most likely be detrimental if it passed up the opportunity to feed on a particular host in order to find one that it preferred.

CHAPTER V

SUMMARY AND CONCLUSION

In this study, single-strand conformation polymorphism was used to detect different haplotypes of the 16S rDNA gene within samples of Gulf Coast ticks collected from Payne County, Oklahoma and in Brazos and Refugio Counties, Texas. The procedure sometimes yielded gels that were difficult to interpret because the banding patterns of the DNA fragments for the different haplotypes only had small variations. In the future, the 16S rDNA gene fragments should be subjected to DNA sequencing, instead of running the fragments on a gel. This would reduce the chances for error in classifying a sample as a specific haplotype and would increase the likelihood of identifying new haplotypes.

Samples of *A. maculatum* were collected from dogs, horses, and cattle. The haplotype frequencies from the collection of cattle were compared to a previous study to detect any changes that had occurred. In the samples from Payne County and Refugio County, haplotype D was the dominating haplotype yet it was not even documented in these counties from the previous study. Possible explanations as to why this haplotype is now so prevalent includes the transfer and dispersal of *A. maculatum* by wildlife and ticks with this haplotype having a higher fecundity or resistance to desiccation and acaricides. It is unknown whether haplotypes fluctuate on a yearly basis or how changes in the environment affect the survival of these ticks, however this provides a foundation for future work to answer these questions. The comparison of ticks from cattle in

Refugio and Brazos County, Texas and Payne County, Oklahoma implied that Refugio County had a unique population structure, in terms of the 16S rDNA gene, when compared to Brazos and Payne Counties. Also, Brazos and Payne Counties had similar haplotype frequencies even though Brazos County did not contain haplotype C. The frequencies of the haplotypes from ticks from the various hosts did not show any differences when they were compared, which suggests that *A. maculatum* does not have any host preferences, that can be identified with this gene, when seeking out a host.

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